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(57) Abstract

This invention relates to newly identified pancreas or pancreatic cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "pancreatic cancer antigens", and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such pancreatic cancer antigens for detection, prevention and treatment of disorders of the pancreas, particularly the presence of pancreatic cancer. This invention relates to the pancreatic cancer antigens as well as vectors, host cells, antibodies directed to pancreatic cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the pancreas, including pancreatic cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of pancreatic cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

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Human Pancreas and Pancreatic Cancer Associated Gene Sequences and Polypeptides

Field of the Invention

This invention relates to newly identified pancreas or pancreatic cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "pancreatic cancer antigens," and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such pancreatic cancer antigens for detection, prevention and treatment of disorders of the pancreas, particularly the presence of pancreatic cancer. This invention relates to the pancreatic cancer antigens as well as vectors, host cells, antibodies directed to pancreatic cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the pancreas, including pancreatic cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of pancreatic cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

Background of the Invention

Cell growth is a carefully regulated process which responds to specific needs of the body. Occassionally, the intricate, and highly regulated controls dictating the rules for cellular division break down. When this occurs, the cell begins to grow and divide independently of its homeostatic regulation resulting in a condition commonly referred to as cancer. In fact, cancer is the second leading cause of death among Americans aged 25-44.

Pancreatic cancer is one of the most dangerous cancers, killing half its victims within 6 weeks and having a 5-year survival rate of only 1%. The diagnosis of pancreatic carcinoma is often associated with a poor prognosis, because most patients already have advanced disease. Despite the many advances reported during the past few years, pancreatic cancer remains a profound therapeutic challenge. It is hoped that the increasing knowledge of the

molecular biology of pancreatic carcinoma will lead to improvements in diagnosing, staging, and treating pancreatic adenocarcinoma (Brand et al., Curr Opin Oncol 10:362-6 (1998)).

There is a need, therefore, for identification and characterization of factors that modulate activation and differentiation of pancreatic cells, both normally and in disease states. In particular, there is a need to isolate and characterize additional molecules that mediate apoptosis, DNA repair, tumor-mediated angiogenesis, genetic imprinting, immune responses to tumors and tumor antigens and, among other things, that can play a role in detecting, preventing, ameliorating or correcting dysfunctions or diseases related to the pancreas.

Summary of the Invention

The present invention includes isolated nucleic acid molecules comprising, or alternatively, consisting of, a pancreas and/or pancreatic cancer associated polynucleotide sequence disclosed in the sequence listing (as SEQ ID NOs:1 to 459) and/or contained in a human cDNA clone described in Tables 1, 2 and 5 and deposited with the American Type Culture Collection ("ATCC"). Fragments, variant, and derivatives of these nucleic acid molecules are also encompassed by the invention. The present invention also includes isolated nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide encoding a pancreas and/or pancreatic cancer polypeptide. The present invention further includes pancreas and/or pancreatic cancer polypeptides encoded by these polynucleotides. Further provided for are amino acid sequences comprising, or alternatively consisting of, pancreas and/or pancreatic cancer polypeptides as disclosed in the sequence listing (as SEQ ID NOs: 460 to 918) and/or encoded by a human cDNA clone described in Tables 1, 2 and 5 and deposited with the ATCC. Antibodies that bind these polypeptides are also encompassed by the invention. Polypeptide fragments, variants, and derivatives of these amino acid sequences are also encompassed by the invention, as are polynucleotides encoding these polypeptides and antibodies that bind these polypeptides. Also provided are diagnostic methods for diagnosing and treating, preventing, and/or prognosing disorders related to the pancreas, including pancreatic cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of pancreatic cancer antigens of the invention.

Detailed Description

Tables

Table 1 summarizes some of the pancreatic cancer antigens encompassed by the invention (including contig sequences (SEQ ID NO:X) and the cDNA clone related to the contig sequence) and further summarizes certain characteristics of the pancreatic cancer polynucleotides and the polypeptides encoded thereby. The first column shows the "SEQ ID NO:" for each of the 459 pancreatic cancer antigen polynucleotide sequences of the invention. The second column provides a unique "Sequence/Contig ID" identification for each pancreas and/or pancreatic cancer associated sequence. The third column, "Gene Name," and the fourth column, "Overlap," provide a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database and the database accession no. for the database sequence having similarity, respectively. The fifth and sixth columns provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity), respectively, observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence. The ninth column provides a unique "Clone ID" for a cDNA clone related to each contig sequence.

Table 2 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, fifteen or more of any one or more of these public EST sequences are optionally excluded from certain embodiments of the invention.

Table 4 lists residues comprising antigenic epitopes of antigenic epitope-bearing fragments present in most of the pancreas and/or pancreatic cancer associated polynucleotides described in Table 1 as predicted by the inventors using the algorithm of Jameson and Wolf, (1988) Comp. Appl. Biosci. 4:181-186. The Jameson-Wolf antigenic analysis was performed using the computer program PROTEAN (Version 3.11 for the Power MacIntosh, DNASTAR, Inc., 1228 South Park Street Madison, WI). Pancreas and pancreatic cancer associated polypeptides (e.g., SEQ ID NO:Y, polypeptides encoded by SEQ ID NO:X,

or polypeptides encoded by the cDNA in the referenced cDNA clone) may possess one or more antigenic epitopes comprising residues described in Table 4. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly. The residues and locations shown in column two of Table 4 correspond to the amino acid sequences for most pancreas and/or pancreatic cancer associated polypeptide sequence shown in the Sequence Listing.

Table 5 shows the cDNA libraries sequenced. and ATCC designation numbers and vector information relating to these cDNA libraries.

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X (as described in column 1 of Table 1) or the related cDNA clone (as described in column 9 of Table 1 and contained within a library deposited with the ATCC). For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously

excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

In the present invention, "SEQ ID NO:X" was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X is deposited at Human Genome Sciences, Inc. (HGS) in a catalogued and archived library. As shown in column 9 of Table 1, each clone is identified by a cDNA Clone ID. Each Clone ID is unique to an individual clone and the Clone ID is all the information needed to retrieve a given clone from the HGS library. In addition to the individual cDNA clone deposits, most of the cDNA libraries from which the clones were derived were deposited at the American Type Culture Collection (hereinafter "ATCC"). Table 5 provides a list of the deposited cDNA libraries. One can use the Clone ID to determine the library source by reference to Tables 2 and 5. Table 5 lists the deposited cDNA libraries by name and links each library to an ATCC Deposit. Library names contain four characters, for example, "HTWE." The name of a cDNA clone ("Clone ID") isolated from that library begins with the same four characters, for example "HTWEP07". As mentioned below, Table 1 correlates the Clone ID names with SEQ ID NOs. Thus, starting with a SEQ ID NO, one can use Tables 1, 2 and 5 to determine the corresponding Clone ID, from which library it came and in which ATCC deposit the library is contained. Furthermore, it is possible to retrieve a given cDNA clone from the source library by techniques known in the art and described elsewhere herein. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made persuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein), and/or sequences contained in the related cDNA clone within a library deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also included within "polynucleotides" of the present invention are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or

RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb. 200 kb. 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

"SEQ ID NO:X" refers to a pancreatic cancer antigen polynucleotide sequence described in Table 1. SEQ ID NO:X is identified by an integer specified in column 1 of Table 1. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF) encoded by polynucleotide SEQ ID NO:X. There are 459 pancreatic cancer antigen polynucleotide sequences described in Table 1 and shown in the sequence listing (SEQ ID NO:1 through SEQ ID NO:459). Likewise there are 459 polypeptide sequences shown in the sequence listing, one polypeptide sequence for each of the polynucleotide sequences (SEQ ID NO:460 through SEQ ID NO:918). The polynucleotide sequences are shown in the sequence listing immediately followed by all of the polypeptide sequences. Thus, a polypeptide sequence corresponding to polynucleotide sequence SEQ ID NO:1 is the first polypeptide sequence shown in the sequence listing. The second polypeptide sequence corresponds to the polynucleotide sequence shown as SEQ ID NO:2, and so on. In otherwords, since there are 459 polynucleotide sequences, for any polynucleotide sequence SEQ ID NO:X, a corresponding polypeptide SEQ ID NO:Y can be determined by the formula X + 459 = Y. In addition, any of the unique "Sequence/Contig ID" defined in column 2 of Table 1, can be linked to the corresponding polypeptide SEQ ID NO:Y by reference to Table 4.

The polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may

contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

The pancreas and pancreatic cancer polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often

advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The pancreas and pancreatic cancer polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

"A polypeptide having functional activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The functional activity of the pancreatic cancer antigen polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to an antibody to

the full length polypeptide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants derivatives and analogs thereof to elicit polypeptide related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Pancreas and Pancreatic Cancer Associated Polynucleotides and Polypeptides of the Invention

It has been discovered herein that the polynucleotides described in Table 1 are expressed at significantly enhanced levels in human pancreas and/or pancreatic cancer tissues. Accordingly, such polynucleotides, polypeptides encoded by such polynucleotides,

and antibodies specific for such polypeptides find use in the prediction, diagnosis, prevention and treatment of pancreas related disorders, including pancreatic cancer as more fully described below.

Table 1 summarizes some of the polynucleotides encompassed by the invention (including contig sequences (SEQ ID NO:X) and the related cDNA clones) and further summarizes certain characteristics of these pancreas and/or pancreatic cancer associated polynucleotides and the polypeptides encoded thereby.

Table 1

Seq ID No.	Sequence/ Contig ID	Gene Name	Overlap	HGS Nucleotide Start	HGS Nucleotide End	% Identity	% Similarity	Clone ID
1	456379			3	197			HCDAJ28
2	462108	5'-AMP-activated protein kinase, gamma-1 subunit [Homo sapiens] >sp|P54619|AAKG_HUMAN 5'-AMP-ACTIVATED PROTEIN KINASE, GAMMA-1 SUBUNIT (AMPK GAMMA-1 CHAIN). Length = 331	gi|1335856	2	1033	100	100	HHPDJ86
3	503446			161	415			HTTEU40
4	507841			1	159			HOAAF52
5	509287	Similarity to Yeast SOH-1 protein (SW:P38633) [Caenorhabditis elegans] >sp|P91869|P91869 F32H2.2 PROTEIN. Length = 163	gnl|PID|e1346272	1	480	66	80	HMTME61
6	509672			16	141			HTPAM31
7	509673			1	162			HMWBR61
8	518767			2	148			HTPBZ88
9	522008			1	378			HDPFM59
10	524112			248	475			HJPCM86
11	525971	clk2-136; putative [Homo sapiens] >pir|S53638|S53638 protein kinase clk2-139 (EC 2.7.1.-) - human Length = 139	gi|632966	1	579	95	97	HTXAR16

296	831500	endothelin 3 precursor [Homo sapiens] >pir|A34378|A34378 endothelin 3 precursor - human >sp|P14138|ET3_HUMAN ENDOTHELIN-3 PRECURSOR (ET-3). Length = 238	gi|182249	2	364	87	87	HWMEM06
297	831501			2	118			HHSB094
298	831502			339	527			HHSC148
299	831508			160	354			HCRMN21
300	831509			450	752			HHSCC33
301	831520			242	424			HHTLE36
302	831547	match: protein P30711 [Homo sapiens] Length = 240	gnl|PID|e313869	2	766	91	92	HTYTA02
303	831548	glutathione transferase T1 [Homo sapiens] >pir|S44358|S44358 glutathione S-transferase Theta - human >sp|P30711|GTT1_HUMAN GLUTATHIONE S-TRANSFERASE THETA 1 (EC 2.5.1.18) (CLASS-THETA). (SUB 2-240) Length = 240	gi|510905	3	257	97	97	HHGDQ55
304	831558			3	410			HHGCU20
305	831847			48	953			HTPE164
306	831893	(AF012023) integrin cytoplasmic domain associated protein; leap-1a [Homo sapiens] >sp|O14713|O14713 INTEGRIN CYTOPLASMIC DOMAIN ASSOCIATED PROTEIN Length = 200	gi|2305238	167	589	78	78	HPJJB54
307	831903			881	1045			HDTEA17

The first column of Table 1 shows the "SEQ ID NO:" for each of the 459 pancreatic cancer antigen polynucleotide sequences of the invention.

The second column in Table 1, provides a unique "Sequence/Contig ID" identification for each pancreas and/or pancreatic cancer associated sequence. The third column in Table 1, "Gene Name," provides a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database, such as GenBank (NCBI). The great majority of the cDNA sequences reported in Table 1 are unrelated to any sequences previously described in the literature. The fourth column, in Table 1, "Overlap," provides the database accession no. for the database sequence having similarity. The fifth and sixth columns in Table 1 provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. In one embodiment, the invention provides a protein comprising, or alternatively consisting of, a polypeptide encoded by the portion of SEQ ID NO:X delineated by the nucleotide position nos. "Start" and "End". Also provided are polynucleotides encoding such proteins and the complementary strand thereto. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity) observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence.

The ninth column of Table 1 provides a unique "Clone ID" for a clone related to each contig sequence. This clone ID references the cDNA clone which contains at least the 5' most sequence of the assembled contig and at least a portion of SEQ ID NO:X was determined by directly sequencing the referenced clone. The reference clone may have more sequence than described in the sequence listing or the clone may have less. In the vast majority of cases, however, the clone is believed to encode a full-length polypeptide. In the case where a clone is not full-length, a full-length cDNA can be obtained by methods described elsewhere herein.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, or more of any one or more of these public ESTs are optionally excluded from the invention.

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing as SEQ ID NO:1 through SEQ ID NO:459) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing as SEQ ID NO:460 through SEQ ID NO:918) are sufficiently accurate and otherwise suitable for a

variety of uses well known in the art and decribed further below. For instance, SEQ ID NO:X has uses including, but not limited to, in designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the related cDNA clone contained in a library deposited with the ATCC. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling immediate applications in chromosome mapping, linkage analysis, tissue identification and/or typing, and a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y have uses that include, but are not limited to, generating antibodies which bind specifically to the pancreatic cancer antigen polypeptides, or fragments thereof, and/or to the pancreatic cancer antigen polypeptides encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing the related cDNA clone (deposited with the ATCC, as set forth in Table 1). The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. Further, techniques known in the art can be used to verify the nucleotide sequences of SEQ ID NO:X.

The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to vectors or plasmids which include such DNA sequences, as well as the use of the DNA sequences. The material deposited with the ATCC on:

Table 2

ATCC Deposits	Deposit Date	ATCC Designation Number
LP01, LP02, LP03, LP04, LP05, LP06, LP07, LP08, LP09, LP10, LP11,	May-20-97	209059, 209060, 209061, 209062, 209063, 209064, 209065, 209066, 209067, 209068, 209069
LP12	Jan-12-98	209579
LP13	Jan-12-98	209578
LP14	Jul-16-98	203067
LP15	Jul-16-98	203068
LP16	Feb-1-99	203609
LP17	Feb-1-99	203610
LP20	Nov-17-98	203485
LP21	Jun-18-99	PTA-252
LP22	Jun-18-99	PTA-253
LP23	Dec-22-99	PTA-1081

each is a mixture of cDNA clones derived from a variety of human tissue and cloned in either a plasmid vector or a phage vector, as shown in Table 5. These deposits are referred to as "the deposits" herein. The tissues from which the clones were derived are listed in Table 5, and the vector in which the cDNA is contained is also indicated in Table 5. The deposited material includes the cDNA clones which were partially sequenced and are related to the SEQ ID NO:X described in Table 1 (column 9). Thus, a clone which is isolatable from the ATCC Deposits by use of a sequence listed as SEQ ID NO:X may include the entire coding region of a human gene or in other cases such clone may include a substantial portion of the coding region of a human gene. Although the sequence listing lists only a portion of the DNA sequence in a clone included in the ATCC Deposits, it is well within the ability of one skilled in the art to complete the sequence of the DNA included in a clone isolatable from the

ATCC Deposits by use of a sequence (or portion thereof) listed in Table 1 by procedures hereinafter further described, and others apparent to those skilled in the art.

Also provided in Table 5 is the name of the vector which contains the cDNA clone. Each vector is routinely used in the art. The following additional information is provided for convenience.

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5.286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res. 16:*7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res. 17:*9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies 5:*58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene.

Vectors pSport1, pCMVSport 1.0, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus 15:*59 (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res. 16:*9677-9686 (1988) and Mead, D. *et al., Bio/Technology 9:* (1991).

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in a deposited cDNA clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include, but are not limited to, preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in the related cDNA clone in the deposit, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or the related cDNA clone (See, e.g., columns 1 and 9 of Table 1). The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the the dDNA in the related cDNA clone contained in a deposited library, are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of, the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the complement of the coding strand of the related cDNA clone contained in a deposited library.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would unduly burden the disclosure of this application. Accordingly, for each "Contig Id" listed in the first column of Table 3, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described in the second column of Table 3 by the general formula of a-b, each of which are uniquely defined for the SEQ ID NO:X corresponding to that Contig Id in Table 1. Additionally, specific embodiments are directed to polynucleotide sequences excluding at least one, two, three, four, five, ten, or more of the specific polynucleotide sequences referenced by the Genbank Accession No. for each Contig Id which may be

included in column 3 of Table 3. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example.

Table 3.

Sequence/ Contig ID	General formula	Genbank Accession No.
456379	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 551 of SEQ ID NO:1, b is an integer of 15 to 565, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where b is greater than or equal to a + 14.	R34554, AA018972, AA055489
462108	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1677 of SEQ ID NO:2, b is an integer of 15 to 1691, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:2, and where b is greater than or equal to a + 14.	T79903, R46289, R73001, R73606, N30140, N35752, W32520, W32636, AA018675, AA018676, AA040600, AA040683, AA070495, AA070381, AA083072, AA134451, AA207060, AA207086
503446	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 466 of SEQ ID NO:3, b is an integer of 15 to 480, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:3, and where b is greater than or equal to a + 14.	
507841	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 594 of SEQ ID NO:4, b is an integer of 15 to 608, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:4, and where b is greater than or equal to a + 14.	R12126, R14285
509287	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 682 of SEQ ID NO:5, b is an integer of 15 to 696, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:5, and where b is greater than or equal to a + 14.	H01699, H94037, N30572, N57219, N64393, N92189, AA035664, AA037022, AA045335, AA045422, AA056367, AA115587
509672	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 278 of SEQ ID NO:6, b is an integer of 15 to 292, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:6, and where b is greater than or equal to a + 14.	
509673	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 348 of SEQ ID NO:7, b is an integer of 15 to 362, where both a and	

	sequence described by the general formula of a-b, where a is any integer between 1 to 606 of SEQ ID NO:303, b is an integer of 15 to 620, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:303, and where b is greater than or equal to a + 14.	H99806, H99813, AA172251, AA468699, AA659754, AA808925, AA837298, AA858110, AA864723, AA954263, F18115, N99864
831558	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 519 of SEQ ID NO:304, b is an integer of 15 to 533, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:304, and where b is greater than or equal to a + 14.	H60157, W57916, W57917, AA056029, AA056047, AA142858, AA211887, AA469104, AA659257, AA662867, AA665372, AA728846, AA933045, F17890, AA090265
831847	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1360 of SEQ ID NO:305, b is an integer of 15 to 1374, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:305, and where b is greater than or equal to a + 14.	
831893	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 654 of SEQ ID NO:306, b is an integer of 15 to 668, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:306, and where b is greater than or equal to a + 14.	
831903	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1032 of SEQ ID NO:307, b is an integer of 15 to 1046, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:307, and where b is greater than or equal to a + 14.	
831921	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1672 of SEQ ID NO:308, b is an integer of 15 to 1686, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:308, and where b is greater than or equal to a + 14.	H52554, H66743, H71667, N32238, N77727, W19857, AA017111, AA074918, AA235917, AA236708
831923	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1412 of SEQ ID NO:309, b is an integer of 15 to 1426, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:309, and where b is greater than or equal to a + 14.	
831959	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b,	

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, and/or the cDNA sequence contained in a cDNA clone contained in the deposit.

The present invention also encompasses variants of the pancreas and pancreatic cancer polypeptide sequence disclosed in SEQ ID NO:Y, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X, and/or a polypeptide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the related cDNA contained in a deposited library or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise or alternatively consist of, a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, under low stringency conditions, to the nucleotide coding sequence in SEQ ID NO:X, the nucleotide coding sequence of the related cDNA clone contained in a deposited library, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which

hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the cDNA in the related cDNA clone contained in a deposited library, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these polypeptides under stringent hybridization conditions, or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be, for example, an entire sequence referred to in Table 1, an ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be

compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identiy are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the lenght of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other

manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence in SEQ ID NO:Y or a fragment thereof, the amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determing the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237- 245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences

truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which less than 50, less

than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, as discussed herein, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, as discussed herein, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more

biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show a functional activity (e.g., biological activity) of the polypeptide of the invention of which they are a variant. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein or fragments thereof, (e.g., including but not limited to fragments encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having a functional activity of a polypeptide of the invention.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA in the related cDNA clone contained in a

deposited library, the nucleic acid sequence referred to in Table 1 (SEQ ID NO:X), or fragments thereof. will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side

chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1

amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), an amino acid sequence encoded by SEQ ID NO:X or fragments thereof, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the pancreas and pancreatic cancer polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers, for example, to a polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in a depostied cDNA clone; or is a portion of a polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited cDNA clone; or is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; or is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, at least about 100 nt, at least about 125 nt or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from, for example, the sequence contained in the cDNA in a related cDNA clone contained in a deposited library, the nucleotide sequence shown in SEQ ID NO:X or the complementary stand thereto. In this context "about" includes the particularly recited value or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 150, 175, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-

400, 401-450, 451-500, 501-550, 551-600, 651-700,701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of SEQ ID NO:X, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g., biological activity) of the polypeptide encoded by the polynucleotide of which the sequence is a portion. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700,701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of the cDNA nucleotide sequence contained in the deposited cDNA clone, or the complementary strand thereto. In this context "about" includes the particularly recited range, or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a

functional activity (e.g., biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in the deposited cDNA clone. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, and/or encoded by the cDNA contained in the related cDNA clone contained in a deposited library. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, an amino acid sequence from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, and 1181 to the end of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained

when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, polypeptide fragments of the invention include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in the related cDNA clone contained in a deposited library). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular

polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in deposited cDNA clone referenced in Table 1). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y), and/or the cDNA in the related cDNA clone contained in a deposited library, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X, or encoded by the cDNA in the related cDNA clone contained in a deposited library may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X, or the cDNA in a deposited cDNA clone may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; http://www.dnastar.com/).

Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions,

Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Preferred polypeptide fragments of the invention are fragments comprising, or alternatively consisting of, an amino acid sequence that displays a functional activity of the polypeptide sequence of which the amino acid sequence is a fragment.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of

SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Table 4.

Sequence/ Contig ID	Epitope
462108	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 461 as residues: Ile-1 to Arg-9, Val-26 to Val-41, Met-46 to Cys-51, Trp-88 to Gln-93, Glu-124 to Trp-130, Gly-339 to Pro-344.
503446	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 462 as residues: Leu-54 to Leu-60.
507841	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 463 as residues: Tyr-39 to Trp-44.
509287	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 464 as residues: Arg-6 to Val-12, Thr-38 to Asn-43, Arg-69 to Asp-74, Trp-87 to Lys-97, His-136 to Met-142, Ala-149 to Lys-160.
509672	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 465 as residues: Ser-33 to Cys-39.
524112	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 469 as residues: Asp-1 to Gly-6, Pro-30 to Gly-40, Leu-46 to Asn-52, Asp-54 to Gly-61.
525971	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 470 as residues: Pro-13 to Arg-21, Leu-30 to Thr-35, Pro-43 to Ser-51.
527156	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 471 as residues: Ala-2 to Pro-7.
532502	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 472 as residues: Lys-1 to Ser-6.
533459	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 473 as residues: Gly-1 to Trp-7, Ile-155 to Gly-163.
533551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 474 as residues: Lys-15 to Leu-20.
537850	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 475 as residues: Ile-43 to Leu-49, Cys-85 to Lys-92, Phe-138 to Leu-144.
537925	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 476 as residues: Gln-17 to Ser-24, Ala-47 to Asn-52.
540802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 479 as residues: Leu-3 to Trp-9, Arg-20 to Phe-29, Glu-58 to Gln-65.
540989	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 480 as residues: Ser-52 to Gly-57, Thr-64 to Asn-70.
540997	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 481 as residues: Ile-1 to Thr-11.
548735	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 482 as residues: Gln-17 to Asn-22, Ser-38 to Pro-45, Asn-75 to Leu-84, Glu-97 to Pro-110.
549709	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 483 as residues: Phe-65 to Trp-77.
550007	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 484 as residues: Ser-4 to Ser-13, Leu-22 to Cys-40, Gly-42 to Gly-50, Thr-88 to Glu-97, Leu-184 to Gln-190, Pro-206 to Gly-211.
550118	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 485 as residues: Gly-1 to Gly-7, Trp-10 to Met-24, Gln-91 to Gly-98.
550870	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 487 as residues: Arg-26 to Arg-33, Gln-47 to Asn-52, Trp-61 to Ser-71, Gly-93 to Trp-100.
553765	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 489 as residues: Thr-8 to Thr-19, Arg-108 to Ser-115, Ser-117 to Arg-128, Phe-143 to Tyr-155, Leu-171 to Arg-177, Asn-182 to Gly-187, Gly-195 to Ser-200, Arg-232 to Thr-

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(c) a polynucleotide encoding a polypeptide fragment of a polypeptide encoded by SEQ ID NO:X or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(d) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(e) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(f) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X, having biological activity;

(g) a polynucleotide which is a variant of SEQ ID NO:X;

(h) a polynucleotide which is an allelic variant of SEQ ID NO:X;

(i) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;

(j) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide

sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(b) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(d) a polypeptide epitope of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(e) a full length protein of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(f) a variant of SEQ ID NO:Y;

(g) an allelic variant of SEQ ID NO:Y; or

(h) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

SEQUENCE LISTING

<110> Craig Rosen,
Steve Ruben

<120> Human Pancreas and Pancreatic Cancer Associated Gene Sequences and Polypeptides

<130> PA105PCT

<140> Unassigned
<141> 2000-03-08

<150> 60/124,270
<151> 1999-03-12

<160> 928

<170> PatentIn Ver. 2.0

<210> 1
<211> 565
<212> DNA
<213> Homo sapiens

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tctcaccaaa cctgctggga atgaatccta catatatatt tatatgtgtg caggctacat 180
ggttttcatt atgctattga tkttaaaagt aaaaatttgg gaaagaacct ccatgtccac 240
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ctactgattt ccttgtagat attttgaaac agataagaaa ctcctccgtg gaaattactg 480
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aaaacttgga aaatgcataa aagta 565

<210> 2
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<212> DNA
<213> Homo sapiens

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agatagctcc ccagctgtgg aaaatgagca tcctcaagag accccagaat ccaacaatag 120
cgtgtatact tccttcatga agtctcatcg ctgctatgac ctgattccca caagctccaa 180
attggttgta tttgatacgt ccctgcaggt gaagaaagct ttttttgctt tggtgactaa 240

cggtgtacga gctgcccctt tatgggatag taagaagcaa agttttgtgg gcatgctgac 300
catcactgat ttcatcaata tcctgcaccg ctactataaa tcagccttgg tacagatcta 360
tgagctagaa gaacacaaga tagaaacttg gagagaggtg tatctccagg actcctttaa 420
accgcttgtc tgcatttctc ctaatgccag cttgtttgat gctgtctctt cattaattcg 480
gaacaagatc cacaggctgc cagttattga cccagaatca ggcaatactt tgtacatcct 540
cacccacaag cgcattctga agttcctcaa attgtttatc actgagttcc ccaagccaga 600
gttcatgtcc aagtctctgg aagagctaca gattggcacc tatgccaata ttgctatggt 660
tcgcactacc acccccgtct atgtggctct ggggatttttt gtacagcatc gagtctcagc 720
cctgccagtg gtggatgaga aggggcgtgt ggtggacatc tactccaagt ttgatgttat 780
caatctggca gcagaaaaga cctacaacaa cctagatgta tctgtgacta aagccttgca 840
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aaaaaaaaaa a 1691

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cccttacaat agtcattcca tccatctgtt gcttatttga attctcattt atttttactt 180
tatggcatta aaatacaata aatctgtcaa ttatgtattt tatattagta gtagcttaag 240
attgggtcac ttcatttcgg tagatataat tgttagtatt atccttcagg acaaaaagca 300
tctgctaaca acctgtggtt taaaatatag gccaacttta tgttcaaaca ttatgttgat 360
aatattttta gcagtattac acagtggagg tccaaattgg attagacttt tgcattgatt 420
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<210> 4
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<213> Homo sapiens

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<220>
<221> misc feature
<222> (578)
<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (582)
<223> n equals a,t,g, or c

<400> 4
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aaatggtact ggrtcttagt ttctgtgcag aatattctgt gaattttggg aaatgtaagt 180
gagtccactt gcttctggac caattctgtt tcatgtatgt tagcatccta gaaacaccta 240
gcaatggacc tagttcacag taataggtgc aaagaaagac caaatggact ttgcagtatt 300
aacccтttgc agtcgtcata cttagctgct gcctgtaatg ctaaaatgat tttaatggtt 360
gtctggaggc aaagggctgt tttttagtat attgccacta aaggacattt atttatatca 420
aacttttatt tttagatatt ataagcatac agtacataat tgatgaaatt gatatttact 480
agagatttat ggtagagaat ggacgacatt caataactgg gagcccgaga ttgtycactt 540
tattttaaaa agacaaataa tcmnctggac aagacagnca cngttggcca ttataaggga 600
gaaatagg 608

<210> 5
<211> 696
<212> DNA
<213> Homo sapiens

<400> 5
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gctggaaatc gacttcggtt tcagttggag ttggaatttg tgcaatgttt agccaaccca 180
aattacctta attttcttgc ccaaagaggt tacttcaaag acaaagcttt tgttaattat 240
cttaaatact tgctttactg gaaagaccca gaatatgcca agtatctaaa gtaccctcag 300
tgtttacaca tgttagagct gctccaatat gaacacttcc gaaaggagct ggtgaatgct 360
cagtgtgcga aatttattga tgaacagcag attctacatt ggcagcacta ttcccggaag 420
cggatgcgcc ttcagcaagc cttggcagag cagcaacagc aaaataacac atcgggaaaa 480
tgaaaaactg gatacaaacg aggcacttaa tacatgtata taatgtattt cttttgtaca 540
gtgaagacaa aaaaaatgaa aactcttcct atctaccttt attatggtag cccctagaac 600
ctttagtgtg ctttttcatc caacttttta ttgttaatag actatttctg ttaaacctga 660
attgttcttt gatttccctg tggatttgta aggtgt 696

<210> 6
<211> 292
<212> DNA
<213> Homo sapiens

<222> (575)
<223> n equals a,t,g, or c

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tgggcgccaa aggccgcggc actcccacgc ggaccccgaa gtccgcaacc cggggatggg 120
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gggcaaaaaa cgacacagta gtagcagttc ccaaagtagc gaaatcagta ctaagagcaa 240
gtctgtggat tctagccttg ggggtctttc acgatccagc actgtggcca gcctcgacac 300
agattccacc aaaagctcag gacaaagcaa caataattca gatacctgtg cagaatttcg 360
aataaaatat gttggtgcca ttgagaaact gaaactctcc gagggaaaag gccttgaagg 420
gccattagac ctgataaatt atatagacgt tgcccagcaa gatggaaagt tgccttttgt 480
tcctccggag gaagaattta ttatgggagt ttccaagtat ggcataaaag tattcaacat 540
cagrtcaata tgtaagtnat ataatttatt aaganaacta tgttttagat aacagggaat 600
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gtaaacat 668

<210> 307
<211> 1046
<212> DNA
<213> Homo sapiens

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<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (14)
<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (946)
<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (948)
<223> n equals a,t,g, or c

<400> 307
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gaagcataca taaataaatg aagtaagcca tactgattta atttattgga tgttattttc 120
cctaagacct gaaaatgaac atagtatgct agttattttt cagtgttagc cttttacttt 180
cctcacacaa tttggaatca tataatatag gtactttgtc cctgattaaa taatgtgacg 240
gatagaatgc atcaagtgtt tattatgaaa agagtggaaa agtatatagc ttttagcaaa 300
aggtgtttgc ccattctaag aaatgagcga atatatagaa atagtgtggg catttcttcc 360
tgttaggtgg agtgtatgtg ttgacatttc tccccatctc ttcccactct gttttctccc 420
cattatttga ataaagtgac tgctgaagat gactttgaat ccttatccac ttaatttaat 480

gtttaaagaa aaacctgtaa tggaaagtra gactccttcc ctaatttcag tttagagcaa 540
cttgaagaag agtagacaaa aaataaaatg cacatagaaa aagagaaaaa gggcacaaag 600
ggattggccc aatattgatt ctttttttata aaacctcctt tggcttagaa ggaatgactc 660
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tccaccttta ggtattttag agcacagaac aacactgtgt tgatctagta ggtttctatt 780
tttccttttct ctttacaatg cacataatac tttcctgtat ttatatcata acgtgtatag 840
tgtaaaatgt gaatgacttt ttttgtgaat gaaaatctaa aatctttgta acttttttata 900
tctgcttttg tttcaccaaa gaaacctaaa atccttcttt tamwananaa aaaaaaaaaa 960
aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1020
aaaaaaaaaa aagggcggcc gtttta 1046

<210> 308
<211> 1686
<212> DNA
<213> Homo sapiens

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<223> n equals a,t,g, or c

<220>
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<222> (29)
<223> n equals a,t,g, or c

<220>
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<222> (39)
<223> n equals a,t,g, or c

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<222> (117)
<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (1522)
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<220>
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<222> (1551)
<223> n equals a,t,g, or c

<220>
<221> misc feature
<222> (1627)
<223> n equals a,t,g, or c